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Cell, Vol. 95, 733–735, December 11, 1998, Copyright ©1998 by Cell Press

# Y Do We Drink?

Minireview

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Consumption of alcohol, in the form of fermented fruit juices, honey, or grains, is probably as old as mankind. Indeed, for nearly 10,000 years of recorded Western history, beer and wine were the primary beverages used to quench thirst (Vallee, 1994). Water was considered to be poisonous and cause a variety of acute and chronic illnesses, and even death; the role of various waterborne pathogens in disease was not recognized until the 19th century. It is thus possible that a state of mild intoxication influenced many people's state of mind, providing some relief from the hardships of life. These alcoholic beverages also provided substantial calories and essential vitamins and minerals and were therefore an important component of the diet. While there are multiple allusions to excessive consumption throughout history, it wasn't until the advent of alcohol distillation, and the consequent availability of spirits with high-alcohol content, that the negative effects of alcohol on individuals and societies became increasingly obvious (Vallee, 1994, and references therein). Current epidemiological data show that the prevalence of alcohol abuse and dependence in the USA is 7%–8% and that the economic cost for alcohol-related problems may exceed $125 billion.

Why do we drink? Most youths experiment with alcohol due to multiple factors such as curiosity and peer pressure. However, most people who drink do not become addicted. What determines the transition from social or moderate drinking to abuse and addiction? There is ample evidence from family, twin, and adoption studies that genetic as well as environmental factors contribute to the risk for alcoholism, but to date no single gene has been conclusively associated with an increased risk. Rather, it is likely that multiple genes interact in complex ways with each other and with individuals' unique life experiences to determine susceptibility for alcohol addiction. Moreover, different sets of genes may contribute to this risk in different people. Thus, despite major advances in our understanding of the neurobiological mechanisms of alcohol's action in the brain, the causes of alcohol addiction remain an enigma. Alcohol research has been confounded by the complexity of the clinical phenotype, the complexity of addiction genetics, and by the fact that ethanol does not appear to have a specific target in the brain, but rather modulates the function of multiple neurotransmitter systems, such as $GABA_A$ and NMDA, and voltage-gated ion channels. Here we discuss recent findings that implicate a new player, neuropeptide Y (NPY), in the regulation of mammalian responses to alcohol. Specifically, Thiele et al. (1998) show that genetic manipulations that alter the levels of NPY in mice affect the sensitivity of these animals to the sedative effects of ethanol as well as their preference for ethanol. We consider these observations in the context of proposed roles for NPY in regulating diverse behaviors and discuss how they may stimulate new investigations into the neurobiological basis of alcohol addiction.

### *Ethanol-Preferring and -Nonpreferring Rats*

Rodent models, accessible to genetic and pharmacological analyses, have been used widely to study complex alcohol-related behaviors (reviewed in Crabbe et al., 1994). The analysis of NPY as a potential regulator of ethanol consumption in mice was inspired by recent findings obtained with a well-studied rat model of alcoholism, the ethanol-preferring (P) rats, which were derived by selective breeding for increased ethanol consumption (reviewed in Li et al., 1993). Specifically, rats were provided a choice of two bottles, one containing a 10% ethanol solution and the other water; those rats ingesting high and low amounts of alcohol were bred for 30 generations to obtain the P and NP (nonpreferring) strains, respectively. Inbred P rats not only voluntarily consume approximately 10-fold more alcohol than NP rats, but also self-administer the drug (orally, intragastrically, or intracerebrally) to the point of obvious intoxication. In addition, intravenous infusion of relatively low ethanol doses curtails voluntary intake in P rats. These data argue that P rats self-administer ethanol for its pharmacological effects rather than its smell, taste, or caloric content. P rats are also more resistant to the sedative effects of alcohol; they develop tolerance when allowed chronic free-choice drinking and display signs of physical dependence upon withdrawal of the drug. Perhaps more importantly, P rats show higher operant responding for ethanol than NP rats (in other words, they are more willing to work for their alcohol), indicating a genetically determined difference in the reward value of alcohol. In summary, P rats, which were bred exclusively for increased ethanol preference by oral self-administration, display multiple alcohol-related behaviors commonly associated with human alcohol addiction.

In order to begin a genetic and molecular understanding of these rats, quantitative trait locus (QTL) analysis was carried out in a two-generation intercross between P and NP rats. A QTL with a major effect on alcohol consumption was mapped to a region of chromosome 4 (Carr et al., 1998). This region contains, among many others, the gene encoding the precursor of NPY. Whether particular *NPY* alleles modulate the ethanol-drinking behavior of P/NP rats remains to be resolved, but circumstantial evidence makes *NPY* a good potential candidate gene. For example, P rats express reduced levels of NPY in brain regions implicated in emotional responses (Ehlers et al., 1998). The enhancement of anxiety-like behaviors in P rats (Steward et al., 1993) is therefore consistent with proposed anxiety-reducing properties of NPY (see below). However, whether low NPY levels

and high anxiety are causative factors in the ethanol-preference of P rats remains to be determined. Additional QTLs contributing to ethanol preference have also been mapped in mice (Phillips et al., 1994; Melo et al., 1996).

### *Ethanol Intoxication and Preference in the NPY Mutant Mouse*

Recently, Todd Thiele teamed up with the Palmiter laboratory to explore the role of NPY in regulating ethanol-related behaviors more directly (Thiele et al., 1998). NPY knockout (NPY-KO) mice and mice overexpressing NPY (NPY-OX mice) were tested for ethanol preference and for sensitivity to the acute sedative/hypnotic effects of ethanol. When presented a choice between water and an ethanol solution, NPY mutant mice consumed almost twice as much alcohol as control mice at ethanol concentrations of 6%, 10%, and even 20% (about the levels found in beer, wine, and some spirits, respectively). Moreover, the NPY-KO mice showed a strong preference for ethanol over water; when tested with 6% ethanol, nearly 70% of their total liquid intake derived from the alcohol-containing bottle. Under these experimental conditions, no differences were observed for food and total liquid consumption or for preference for sweet (sucrose) and bitter (quinine) solutions. Thus, the enhanced ethanol preference displayed by NPY-KO mice was not simply due to changes in fluid consumption, caloric need, or taste novelty. NPY-KO mice were also less sensitive than controls to the sedative effects of an acute dose of alcohol. In the loss-of-righting-reflex (LORR) assay, the time required for the animal to restore normal posture was recorded after an intraperitoneal injection of a sedative alcohol dose. Inebriated NPY-KO mice were able to right themselves significantly faster than controls. This difference could not be accounted for by changes in ethanol pharmaco-kinetics as these mice displayed normal ethanol clearance. In summary, the NPY-KO mice exhibited two ethanol responses that are similar to those of P rats, in that they consume more alcohol and are less sensitive to its sedative effects than their respective controls. Similarly, mice lacking the 5-HT1B serotonin receptor display reduced sensitivity to ethanol-induced ataxia and elevated ethanol consumption (Crabbe et al., 1994). More importantly, the sensitivity of humans to the acute effects of alcohol appears to be genetically influenced, and increased resistance is a good predictor of risk for alcoholism (Schuckit, 1994). However, whether ethanol resistance and preference (or addiction) are causally related is unknown.

To test the converse, that is whether increased levels of NPY lead to reduced ethanol-consumption, Thiele et al. (1998) examined transgenic mice overexpressing NPY by about 5-fold in brain regions (including cortex, amygdala, and hippocampus) where NPY is normally expressed. These NPY-OX mice drink significantly less alcohol than their wild-type littermates and are more sensitive to the sedative effects of ethanol in the LORR assay. NPY-OX mice do not differ from controls in their consumption of food, total liquid intake, or their ability to metabolize ethanol. Thus, there is a striking bidirectional inverse relationship between NPY levels and ethanol preference and resistance.

### *NPY, Feeding, and Foraging*

The findings described above with the NPY-KO and NPY-OX mice reveal a novel role for a neuropeptide system that has already been implicated as an important modulator of central nervous system physiology and behavior. NPY is a 36–amino acid peptide member of a family of polypeptides that includes peptide YY and pancreatic polypeptide. Whereas the latter two family members are found primarily in the gastrointestinal system, NPY is abundantly expressed in the central and peripheral nervous systems. Expression levels are particularly high in the basal ganglia, cerebral cortex, hypothalamus, amygdala, and other limbic system structures (reviewed in Heilig et al., 1994). NPY acts through several receptors that belong to the G protein–coupled receptor superfamily; the known NPY receptor subtypes inhibit adenylate cyclase and the accumulation of cAMP through pertussis toxin–sensitive G proteins (Blomqvist and Herzog, 1997).

Considerable attention has been paid to the contribution of NPY to the regulation of "basic vegetative functions." Among these, the dramatic effects of NPY on feeding behavior and energy balance have been well studied. Hypothalamic administration of NPY induces food seeking and excessive feeding, even in animals that had recently consumed meals; continuous administration leads to the rapid development of obesity. In further support for a role of NPY as an important modulator of appetite, fasting was found to increase hypothalamic NPY expression, an effect reversed by refeeding (reviewed in Heilig et al., 1994). Independent of its effects on food intake, NPY increases insulin secretion and reduces firing rates of sympathetic nerves that stimulate heat production (Frankish et al., 1995). Thus, NPY appears to be a potent modulator of food intake and of physiological processes that promote a positive energy balance.

As expected for an abundant neuropeptide with a widespread anatomical distribution, NPY appears to influence multiple behavioral and physiological processes. For example, in several rodent anxiety models, NPY produces apparent anxiety-reducing effects that are independent of alterations in appetite (reviewed in Heilig et al., 1994). NPY receptors in the amygdala may mediate these effects, as indicated by the anxiety-reducing effects of NPY injections into this structure. In addition to the modulation of feeding and emotional responses, NPY applied to the spinal cord produces potent analgesic effects (Hua et al., 1991). One may therefore speculate that in a natural setting, multiple effects of NPY—stimulation of hunger, reduction of anxiety, and diminished pain sensitivity—may promote foraging behavior and the acquisition of food. Interestingly, genetic variation in an NPY receptor homolog modulates foraging behavior in the nematode *C. elegans* (de Bono and Bargmann, 1998). It is thus possible that NPY pathways may have evolved to coordinate a complex set of neurobehavioral responses to attain positive energy balance.

In light of these findings, feeding studies in NPY null mutant mice produced a great surprise: mutants were indistinguishable from wild-type animals with regard to baseline food intake, body weight, and responses to fasting (Erickson et al., 1996). This result raises the possibility that the prominence of NPY in feeding regulation may have been overestimated. However, well-known caveats to the interpretation of behavioral phenotypes

in mutant mice must also be considered (Wehner and Bowers, 1995). For example, it is possible that neurodevelopmental abnormalities unique to the mutants may have minimized the impact of the mutation. Alternatively, the regulation of a behavior so vital to the survival of the organism may involve multiple redundant neural systems capable of compensating for such genetic lesions.

***Role of NPY in Reward Systems***

The observation of increased ethanol preference in NPY-KO mice raises the possibility that the regulation of reward pathways may be included among the many actions of this neuropeptide. This possible role of NPY had received little attention despite the expression of NPY and its Y1 and Y2 receptor subtypes in the amygdala and nucleus accumbens. These structures are components of the mesolimbic dopamine system, a pathway believed to mediate the rewarding aspects of food, alcohol, and other drugs of abuse (reviewed in Koob, 1992; Heilig et al., 1994). For example, NPY facilitates dopamine release in the nucleus accumbens (Ault et al., 1998) and enhances the extent to which animals will work to receive food rewards (Jewett et al., 1992). Moreover, local injections of NPY into the nucleus accumbens may be rewarding, as indicated by a preference of animals for the environment in which the effects of such injections were experienced (Josselyn and Beninger, 1993). Based on these findings, one might predict that the rewarding effects of alcohol would be blunted in NPY mutant mice.

How, then, can we account for the elevated ethanol preference of mice lacking NPY? Although this result could reflect an increased sensitivity of mutants to the rewarding properties of alcohol, the converse interpretation, diminished sensitivity to ethanol reward, must also be considered. In the latter case, elevated ethanol consumption may be required for animals to obtain pharmacological effects equivalent to those of wild-type mice. It is also important to consider the possibility that animals were not drinking to intoxication during the preference assay. It is known that factors independent of the pharmacological effects of ethanol, such as taste and novelty, may influence preference (Cicero, 1980).

Thus, further studies will be required to clarify the mechanisms underlying the increased preference displayed by the mutant mice. For example, detailed analysis of diurnal patterns of ethanol ingestion will be helpful here. Overconsumption in the form of intermittent prolonged drinking bouts would be more likely to produce pharmacological effects than a diffusely elevated pattern of ingestion. Ultimately, blood alcohol determinations during preference testing will be required to determine whether animals attain levels sufficient to achieve pharmacological effects. In addition, operant conditioning procedures in which animals work for ethanol access will clarify the impact of the *NPY* gene mutation on the rewarding properties of ethanol.

As for all null mutant models, it will be important to determine the extent to which the adult mutant phenotype reflects the normal role for a gene product in the mature nervous system, rather than an indirect consequence of perturbed development. In this case, supporting evidence for the former possibility is provided by the observation of reduced ethanol preference and elevated sensitivity to the sedative effects of ethanol in NPY-overexpressing transgenic mice. The development of NPY receptor–selective agonists and antagonist compounds will provide complementary pharmacological approaches for assessing the modulatory effects of NPY systems on alcohol intake and for determining the identity and anatomical location of the NPY receptors mediating these effects. The development of new mouse lines designed for region-specific and inducible regulation of an *NPY* gene mutation will also aid these efforts.

In summary, the findings described above illustrate the power of genetic methods to provide novel entry points into the biology of complex neural systems. In particular, this work has succeeded in focusing attention on a potentially important neuropeptide system for the regulation of alcohol intake. Future studies that build on these findings may provide the exciting prospect for novel strategies for the treatment of alcoholism.

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